CYTOTHERAPEUTICS, CYTOTHERAPEUTIC UNITS AND METHODS FOR TREATMENTS USING THEM

FIELD OF THE INVENTION

This Application claims priority to U.S. Provisional Application 60/429,702 filed on November 26, 2002. The present invention is directed to improvements in therapeutics utilizing cytotherapeutic formulations. Cytotherapeutic therapy involves the introduction of immature cells, especially stem cells, into a patient in order to secure palliation, amelioration or cure of a disease state. The present invention is also directed to improved cytotherapeutic agents, to methods of producing them, to unit dosage forms of such agents and to novel paradigms for administering cytotherapeutic units to patients in need of therapy.

BACKGROUND OF THE INVENTION

It has been known heretofore to administer certain types of stem cells to humans and to animals in order to achieve a therapeutic end. Much of this has been done with stem cells from adults, such as those found in adult bone marrow, especially for the repopulation of depopulated interosseous spaces, which attend aggressive chemotherapy or radiation therapy, e.g., for treatment of certain cancers. Indeed, such cytotherapy has become relatively widespread and has achieved a level

of success despite limitations including the lack of standardization as to cell numbers and types.

Many of these therapeutic regimes employ relatively mature cellular preparations, e.g. bone marrow. While these have a level of therapeutic potential, such cells possess quite a large number of surface antigens and require immunosupression attendant to administration. Additionally, most cells extracted from adult bone marrow are limited in the types of cells into which they can differentiate. There have been a number of reports that have indicated that most stem cells isolated from adult bone marrow are only able to differentiate into blood cells. While this is useful for the treatment of blood related diseases, e.g., leukemia, these cells are not very useful for treating other types of diseases that are localized to a specific type of tissue or organ. An additional problem with bone marrow preparations is that the process of extracting the marrow is often very painful, and although potential donors can be identified many do not consent to the procedure because of the potential for pain and discomfort.

Recently, cytotherapy employing less mature stem cells, such as, for example, those found in neonatal cord blood, has found some success. However, stem cell preparations from most sources, including from neonatal cord blood, include a diverse population of cells with differing potentials for effective therapy and often do not contain a sufficient number of cells for an optimized therapeutic dose, particularly for an averaged size adult undergoing a transplant for leukemia, for example. It is believed that different scientific and medical groups likely achieve differing preparations with differing characteristics, even when supposedly following the same or similar protocols. Presently, most independent preparations, even those done by the same individual, can have different compositions with the specifics of the

compositions undetermined. In short, there is a complete lack of unit to unit reproducibility and little standardization in the cellular units used in transplants.

The foregoing practices can give rise to inconsistent therapeutic outcomes from different research and medical centers and make accurate, statistical analyses for cytotherapeutic procedures difficult or impossible to attain. There is, thus, a long-felt need for improved cytotherapeutic materials and procedures, ones amenable to reproducible outcomes and to scientific analysis. It is also desired to improve specificity of cytotherapeutic treatments and to affect improved efficiencies and outcomes. Importantly, there is also a need for unit to unit reproducibility which may further the ability to collect sufficient data to advance the medical area devoted to cellular therapies. The present invention provides solutions for these and other long–felt needs.

SUMMARY OF THE INVENTION

As used herein, "cytotherapeutic unit" refers to a cell preparation comprising a plurality of potent cells in which at least one cell type has been tailored for a particular patient or particular disease state. Tailoring may include having a minimum number of said cell type or, alternatively, removal of a portion or all of said cell type.

"Potent," with respect to a cell or cell type, means that the cell or cell type is capable of differentiation into at least one type of cell.

"Pluripotent," with respect to a cell or cell type, means that the cell or cell type is capable of differentiation into at least two different types of cells.

"Antigenic determinant" refers to the set of antigenic regions on the surface of a cell.

"Factor" refers to a cell type by reference to its antigenic determinant. Exemplary factors include CD34, CD8, CD10 and the like. A cell or cell preparation may also be considered to be positive or negative in regard to a particular factor by reference to whether or not a particular cell or cell type exhibits the characteristics of that particular factor.

The present invention provides for cytotherapeutic units comprising a plurality of potent cells, the contents of which are known with respect to the identities and numbers of at least some of the potent cells. To ensure that the identities and numbers of at least some of the potent cells are accurate at least one assay is performed. In some preferred embodiments, the provider of the unit certifies the accuracy of the assay. In other embodiments, the potent cells for which the identities and numbers are known are pluripotent cells. The identities of the potent cells preferably reflect the presence or absence of at least one antigenic determinant on the cells. In some embodiments, the cytotherapeutic unit comprises at least some potent cells exhibiting CD34, CD8, CD10, OCT4, CD38, CXCR4, or CD117, for example. In some embodiments some portion of the cells may also exhibit CD33. In some preferred embodiments, the cytotherapeutic unit comprises cells that lack specific antigenic determinants. In other embodiments, at least one identified potent cell that is derived from a source is specifically excluded or removed from the cellular preparation.

In one embodiment of the invention, some or all cells may be characterized by the presence of one or more of the following cell surface markers: CD10+, CD29+, CD34-, CD38-, CD44+, CD45-, CD54+, CD90+, SH2+, SH3+, SH4+, SSEA3-, SSEA4-, OCT-4+, and ABC-p+.

The potent cells may be obtained from fetal cord blood or other fetal tissue. In some embodiments, potent cells are obtained from placenta, especially postpartum placenta, which has been metabolically supported and nurtured. Potent cells are preferably obtained from postpartum placenta perfusate. The present invention also provides for cytotherapeutic units wherein the potent cells are derived from a plurality of sources. In some embodiments, the potent cells are derived from at least two individuals, at least five individuals, or at least ten individuals. In some embodiments, the unit comprises at least one cell that is autologous. In some other embodiments, the unit comprises at least one cell that is exogenous. In some embodiments the unit comprises a chimera of autologous and allogeneic cells. In another embodiment at least some of the cells are genetically modified.

In other embodiments, the plurality of potent cells is selected to render the unit suitable for therapy for an indicated disease state or condition and/or the severity of the condition. In some preferred embodiments, the cytotherapeutic units comprise a minimum number of preselected types of potent cells and may be based, for example, on the weight of the particular patient or that patient's medical status. In some preferred embodiments, the cytotherapeutic unit is assayed to ensure the accuracy of its contents of preselected types of potent cells. In some preferred embodiments, the contents of the preselected potent cells in the cytotherapeutic unit are certified. In other embodiments, the cytotherapeutic unit can be one of a group of substantially identical units wherein the additional units are stored for future transplants so that, if needed, the patient can receive a unit identical to one previously transplanted. Alternatively, the additional like-units may be altered to optimize future transplants for that same patient.

In other embodiments, at least one type of cell is excluded from the cytotherapeutic unit comprising preselected potent cells. The cytotherapeutic unit is preferably certified as to its contents of the preselected potent cells and the absence of the types of cells to be excluded. In other embodiments, the identity and the numbers of a plurality of potent cells being selected to render the cytotherapeutic unit suitable for therapy for an indicated disease state or condition is certified. In some embodiments, the certification is preferably of a plurality of potent cell types, wherein the plurality and the numbers of each of said plurality being selected as well as excluded renders the cytotherapeutic unit suitable for therapy for an indicated disease state or condition.

In some embodiments, the present invention provides for kits for the treatment of a person suspected of having a disease state or condition. The kit preferably comprises a cytotherapeutic unit comprising a plurality of potent cells. In some embodiments, the kit comprises a cytotherapeutic unit wherein at least one type of cell that has been excluded from the cytotherapeutic unit. In some preferred embodiments, the kit comprises potent cells wherein at least some of the potent cells have been identified and counted. In some embodiments, the kit comprises a unit that has been assayed to ensure the accuracy of the identities and numbers of the potent cells. In some more preferred embodiments of the kit, the accuracy of the assay has been certified.

The present invention provides kits for the treatment of a person suspected of having a disease state or condition comprising a cytotherapeutic unit having minimum numbers of identified potent cells and a certification of the potent cell composition. The kits may also contain equipment or devices for administering the unit to the patient, materials for monitoring the administration and other attendant things.

In some embodiments, the present invention provides for cytotherapeutic units comprising cells derived from umbilical cord blood, placenta, or a mixture thereof, wherein at least one type of cell has been removed from the unit. In some embodiments, a plurality of cell types has been removed from the unit.

The present invention provides for a cytotherapeutic unit comprising cells derived from umbilical cord blood, placenta, or a mixture thereof, wherein said cells comprise a plurality of different types. In some embodiments at least some of the different types of cells are separated into components. In other embodiments, the components are recombined into the unit. It is preferred in some aspects of the invention that components are used to supplement a cytotherapeutic unit with a specific potent cell type. The separated components can be frozen separately or otherwise stored prior to recombination. In some other embodiments, the cytotherapeutic unit itself has been placed in a frozen state. In some further embodiments, the separated cell types have been identified and/or counted.

The present invention provides methods of treating a disease in a mammal comprising administering to the mammal a therapeutically effective amount of a composition comprising a cytotherapeutic unit. The unit used to treat the disease state or condition comprises a plurality of potent cells wherein the content of the unit is known with respect to the identities and numbers. At least some of the cells in the unit are assayed to ensure the accuracy of the identities and the numbers of the potent cells. In some preferred embodiments, the cytotherapeutic unit is administered multiple times. In other cases, administering multiple doses of the cytotherapeutic units that are derived from different individuals or sources may be performed. The methods may also comprise administering multiples doses of the cytotherapeutic unit that is derived from one individual.

The present invention provides for cytotherapeutic units comprising a plurality of potent cells with the content of the cytotherapeutic unit being known with respect to the identities and numbers of at least some of the potent cells.

The identities of the potent cells in the cytotherapeutic unit are an aspect of the invention that is important for the reliability and the quality of the unit being used. The potent cells can be identified by any number of methods and based on any set of criteria that a person of ordinary skill may find useful. One such method is to identify the potent cells based on the presence of antigenic determinants on the surface of the cell. Antigenic determinants can be any molecule that is recognizable by an antibody. Some examples of antigenic determinants include polypeptides, lipids, glycoproteins, sugars, and the like. Additionally, the cells may be characterized by the presence of one or more of the following cell surface markers: CD10+, CD29+, CD34-, CD38-, CD44+, CD45-, CD54+, CD90+, SH2+, SH3+, SH4+, SSEA3-, SSEA4-, OCT-4+, and ABC-p+.

Although some potent cells may be identified by the presence of antigenic determinants or by certain expressed factors, it can be equally important to identify a cell based on what antigenic determinants the cell lacks. For example, it is known that the presence of certain determinants may lower the chances of a successful treatment and therefore, a person using the cytotherapeutic unit would want to know that the unit being used lacks certain antigenic determinants. Furthermore, the presence or absence of antigenic factors can aid in determining the maturity level of a particular cell or cell-type. A less mature cell has a wider range of differentiation and is therefore, potentially more useful. Depending on the use of the cytotherapeutic unit, different levels of differentiation of the cells may be required. The identification

of some of the cells enables a person to obtain a unit, that when used, results in a better clinical outcome.

Methods to determine the presence or absence of antigenic factors on or in a cell are well known in the art. These methods include fluorescence activated cell sorting (FACS), Enzyme-Linked Immuno Sorbent Assay (ELISA), western blot, polymerase chain reaction (PCR), reverse-transcribed PCR (RT-PCR), and the like. The precise method or methods used to identify the potent cells is not essential.

Other criteria to identify a cell can be based on the genetic makeup of the cell. Genes play an essential role in everything that occurs in a cell. Because of this fact, a person of ordinary skill in the art may identify a potent cell based on its genes. More specifically, a person of ordinary skill in the art may identify a cell based on the genes that are wild-type, mutant, being expressed, not being expressed, contain polymorphisms, or a combination thereof. As used herein, the term "expressed" means whether or not the gene is being transcribed into RNA or whether a protein is ultimately produced by that gene.

The methods to determine the genetic profile of a cell are well known to those of ordinary skill in the art. Any method used is sufficient, but some examples of methods or techniques that can be used to determine the genetic makeup of a cell include, without limitation, PCR, RT-PCR, northern blot, southern blot, single nucleotide polymorphism (SNP) analysis, gene-chip expression analysis, serial analysis of gene expression (SAGE), nucleotide sequencing, FACS, *in situ* hybridization, and the like.

In some embodiments of the present invention, a cell can be identified by any of the above-mentioned criteria: antigenic determinants, genetic makeup, a combination thereof, or a cell can be identified based upon another set of criteria. In

some embodiments, at least 0.1%, 1%, at least 10 %, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80 %, at least 90%, at least 95%, or about 100% of the cells are identified.

Methods of identification and determining the number of cells are well known in the art , they include but are not limited to using standard cell detection techniques such as flow cytometry, cell sorting, immunocytochemistry (e.g., staining with tissue specific or cell-marker specific antibodies), FACS, magnetic activated cell sorting (MACS), by examination of the morphology of cells using light or confocal microscopy, or by measuring changes in gene expression using techniques well known in the art, such as PCR and gene expression profiling. Additionally, relevant determinations can be made by techniques including, but not limited to, optical and electrooptical properties, morphological imaging methods, optophoresis (www.genoptix.com) microwave spectroscopy (Signature Bioscience www.signaturebio.com) and optical tweezers. Other methods may also be employed.

It is known that specific cell-types or cells having particular antigenic determinants can have a deleterious effect on the success rate of cytotherapy. Therefore, the present invention provides for cytotherapeutic units that have at least one cell type that is excluded. The cell-type that is excluded will not always be the same. In some embodiments, all CD34 positive cells will be excluded. In some other embodiments all CD8 positive cells will be excluded. In some other embodiments multiple cell types are excluded. In some applications, it may be acceptable and convenient to reduce, rather than eliminate, selected cell types to improve therapeutic success. Thus, the term "exclusion" or "elimination" as used in this context preferably means at least about 75% reduction in the number of a certain cell type in a cell preparation. Preferably, at least about 90% reduction is achieved, with at least about

95% reduction being even more preferred. Essentially complete elimination is, of course, most desirable, although the same may be achievable in some cases. The foregoing percentage reductions relate to numbers of cells relative to an original population of such cells using any appropriate assay.

Cell types can be excluded or reduced either by selecting cell-containing units which, naturally do not contain them (or many of them) or by employing a process that specifically removes selected cell-types. It is preferred to exclude cell types having antigenic determinants which are inconsistent with the therapeutic modality planned for the cytotherapeutic unit. For example, but not by way of limitation, T-lymphocytes and mature dendritic cells may be excluded to lower the expectation of graft versus host disease. In the treatment of adrenal leukodysplasia it may be desirable to delete some or all CD8 positive cells.

To be excluded "naturally" means that the preparation of cells that is derived from a source does not contain a specific cell type without further manipulation or contains a very small population of such types. Alternatively, a cell-type can be excluded by a process that is used either before or after the cells are extracted from a source. Processes or methods that are used to exclude a specific cell-type are well known to the art-skilled. Examples of processes or methods include: FACS, centrifugation, immunochromatography, and the like.

In one embodiment, the cells may be sorted using a fluorescence activated cell sorter (FACS). Fluorescence activated cell sorting (FACS) is a well-known method for separating particles, including cells, based on the fluorescent properties of the particles (Kamrach, 1987, Methods Enzymol, 151:150-165). Laser excitation of fluorescent moieties in the individual particles results in a small electrical charge allowing electromagnetic separation of positive and negative particles from a mixture.

In one embodiment, cell surface marker-specific antibodies or ligands are labeled with distinct fluorescent labels. Cells are processed through the cell sorter, allowing separation of cells based on their ability to bind to the antibodies used. FACS sorter particles may be directly deposited into individual wells of 96-well or 384-well plates to facilitate separation and cloning. Reagents for cell surface markers or cluster designated reagents are available from a variety of sources including Becton Dickinson and Cell Pro Inc., for example.

Available reagents include but are not limited to reagents for identifying: CD1a; CD2; CD3; CD4; CD4 (Multi-Clone); CD4 v4; CD5; CD7; CD8 (Leu-2a); CD8 (Leu-2b); CD10 (Anti-CALLA); CD11a (Anti-LFA-1α); CD11b; CD11c; CD13; CD14; CD15; CD16 (Leu-11a, 11b, 11c); CD18 (Anti-LFA-1β); CD19 (Leu-12); CD19(SJ25C1); CD20; CD21(Anti-CR_2); CD22; CD23; CD25(Anti-IL-2R); CD26; CD27; CD28; CD31(Anti-PECAM-1); CD33; CD34(Anti-HPCA-1&2); CD38; CD42a(Anti-gpIX); CD44; CD45(Anti-Hle-1); CD45RA; CD45RO; CD49d(Anti-VLA-α4); CD54; CD56(MY31); CD56(NCAM16.2); CD57; CD58(Anti-LFA-3); CD61; CD62P; CD62L(Leu-8); CD69; CD71; CD80(Anti-BB1/B7); CD95; CD117; CD122(Anti-IL-2R p75); CD123(Anti-IL-3Rα); CD134(Ox40); CD154(CD40L); CD158a; CD161; Lineage Cocktail 1 (lin1) FITC and others now known or hereafter discovered.

Non-cluster designated reagents include: Anti-BrdU; Anti-Cytokeratin (CAM 5.2); Anti-HER-2/neu; Anti-HLA-DP; Anti-HLA-DQ; Anti-HLA-DR; Anti-Hu KIR (NKB1); Anti-IgA_2; Anti-IgD; Anti-IgG; Anti-IgM (Ig Heavy Chain); Anti-Kappa (Ig Light Chain); Anti-Kappa $F(ab')_2$; Anti-Lambda (Ig Light Chain); Anti-Lambda $F(ab')_2$; Anti-P-glycoprotein (P-gp); Anti-TCR α/β-1 (WT31); Anti-TCR-γ/δ-1; PAC-1; Lineage Cocktail 1 (lin1) FITC. The skilled artisan will use those

reagents required for his/her particular needs in order to optimize the desired cytotherapeutic unit or tailor it for a particular patient or use.

In another embodiment, magnetic beads can be used to separate cells. The cells may be sorted using a magnetic activated cell sorting (MACS) technique, a method for separating particles based on their ability to bind magnetic beads (0.5-100μm diameter). A variety of useful modifications can be performed on the magnetic microspheres, including the covalent addition of an antibody which specifically recognizes a cell-solid phase surface molecule or hapten. A magnetic field is then applied, to physically manipulate the selected beads. The beads are then mixed with the cells to allow binding. Cells are then passed through a magnetic field to separate out cells having cell surface markers. These cells can then isolated and re-mixed with magnetic beads coupled to an antibody against additional cell surface markers. The cells are again passed through a magnetic field, isolating cells that bound both the antibodies. Such cells can then be diluted into separate dishes, such as microtiter dishes for clonal isolation, if desired.

Knowing the composition of the cytotherapeutic unit will help fulfill the long-felt need of a reliable and certified cytotherapeutic unit. In addition to the composition of the unit, it can be useful to know the numbers of at least some of the cells in the cytotherapeutic unit. In some embodiments, just the numbers of cells will be known without knowing the specific identity of any of the cells. In some other embodiments, the numbers of cells will be known, but also the numbers of the identified cells will be known. To determine the number of cells in total is well known to those of ordinary skill in the art. Examples of equipment that can be used to count cells are a machine that performs FACS or flow cytometry, or a much simpler piece of equipment, a hemacytometer. Often the number of the cells will be

determined at the same time the identities are determined, but the numbers can also be determined before or after the identities of some of the potent cells are determined. By knowing the number of the cells present in a cytotherapeutic unit this will give a person using the unit the knowledge of what is being administered, something that is sorely lacking in present cytotherapies.

The knowledge of the numbers of total cells and also the numbers of specific cell types in a cytotherapeutic unit can be used to supplement the unit with additional cells or cell types so that a minimum number of cells or a minimum number of a specific cell type can be present in the unit. It is thought that the diverse responses seen in cytotherapy is in part due to the varying number of cells recovered from a source using the cellular preparation techniques in use today.

By identifying and counting the cells this will allow a more thorough analysis of what is required for a successful treatment as well as the ability to perform a thorough and complete analysis on the importance of a specific cell type in a cellular preparation.

Cytotherapeutic units can now be prepared that have a minimum numbers of preselected cells. It is also now possible to ensure that other cell types are excluded from the units. In some embodiments the cytotherapeutic unit will comprise at least about 100 selected potent cells. Such units having at least about 1,000 such cells are preferred, with at least about 10,000 being more preferred. Greater numbers of selected cells are still more preferred, especially when it is intended that the unit be administered to the same or different individuals a plurality of times. Thus, selected cell populations greater than about 100,000 or even about 500,000 can be useful. It is preferred that some or all of the cells in the unit be identified through assay and that

the same be reflected in a certification of such presence. This certification ensures uniform and effective therapeutic application.

In some embodiments of the present invention, the cytotherapeutic units will have a minimum number of different, specific cell types. Advantages to having a minimum number of specific cell types are that it may improve the efficacy of the cytotherapeutic unit. For example, the cytotherapeutic unit could be assayed to comprise at least about 1,000 OCT4 positive cells, either with or without known quantities of other desirable cell types. In other embodiments, the unit may be caused to comprise specific percentages of CD34 positive cells, measured by reference to all nucleated cells in the preparation. Thus, such preparations may contain at least 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70 %, 80%, 90%, 95% or other percentages of CD34 positive cells may be made. Similar, known percentages of cells having other antigenic determinants or specific factors may, likewise, be created.

Other embodiments of the present invention provide for cytotherapeutic units comprising cells that have been derived from at least one source, wherein the source's cells have been separated into components. As used herein, the term "components" is synonymous to cell-types, identified cells, and the like. Methods to separate cellular preparations into components that are well known to those of skill in the art include, without limitation, FACS, centrifugation, chromatography, HPLC, FPLC, and the like.

Thus, cytotherapeutic units can comprise components that are recombined. In some embodiments, at least one component is used in a cytotherapeutic unit. In some other embodiments, at least two, at least three, at least 4, at least 5, at least 10, at least 100 components are recombined to make the cytotherapeutic unit. It is preferred that

the components of each source be known in terms of identity and relative numbers, with some cell types preferably being excluded from some or all of the components. It may be seen that the different components may be maintained separately, e.g. frozen, and that the same may form a "formulary" or "library" of cells of known identity and abundance for formulation into combined cytotherapeutic units. Separating the respective cellular preparations into components allows a cytotherapeutic unit to be created that has a specific composition both in terms of cells present and in types of cells excluded. Additionally, this allows an existing cytotherapeutic unit to be supplemented with a specific cell-type or component as may be indicated for a specific therapeutic modality.

Thus, cytotherapeutic units of the invention may be seen to comprise cells derived from one source or from many sources. Contrary to prevailing practice, it is believed that there are great benefits to providing cells from a plurality of sources and that therapeutic benefit and efficacy will derive therefrom. In some embodiments, the cells are derived from multiple sources and may derive from multiple organs in such sources. As used herein, the term "source" refers to any organism, tissue, or organ from which cells are derived or extracted. In some embodiments, the sources are fetal cord blood, fetal tissue, placenta, postpartum placenta, postpartum placenta perfusate, or a mixture thereof. It is well known to those of ordinary skill how to extract cells from different tissues or organs. Methods to extract cells from fetal cord blood can be found in, for example in U.S. Patent No. 5,372,581, entitled "Method and apparatus for placental blood collection," issued December 13, 1994; Hessel *et al.,* U.S. Patent No. 5,415,665, entitled "Umbilical cord clamping, cutting, and blood collecting device and method", issued May 16, 1995. The needle or cannula is usually placed in the umbilical vein and the placenta is gently massaged to aid in draining cord blood

from the placenta. Methods to extract cells from placenta, post-partum placenta, or post-partum placenta perfusate can be found in, for example, International Patent Publications WO 02/46373 and WO 02/064755, each of which are herein incorporated by reference in their entireties.

In another embodiment, the cells are stimulated to proliferate, for example, by administration of erythropoietin, cytokines, lymphokines, interferons, colony stimulating factors (CSF's), interferons, chemokines, interleukins, recombinant human hematopoietic growth factors including ligands, stem cell factors, thrombopoeitin (TPO), interleukins, and granulocyte colony-stimulating factor (G-CSF) or other growth factors.

In another embodiment, cells are genetically engineered, for example, using a viral vector such as an adenoviral or retroviral vector, or by using mechanical means such as liposomal or chemical mediated uptake of the DNA.

A vector containing a transgene can be introduced into a cell of interest by methods well known in the art, e.g., transfection, transformation, transduction, electroporation, infection, microinjection, cell fusion, DEAE extran, calcium phosphate precipitation, liposomes, LIPOFECTIN™, lysosome fusion, synthetic cationic lipids, use of a gene gun or a DNA vector transporter, such that the transgene is transmitted to daughter cells, e.g., the daughter embryonic-like stem cells or progenitor cells produced by the division of an embryonic-like stem cell. For various techniques for transformation or transfection of mammalian cells, see Keown *et al.*, 1990, Methods Enzymol. 185:527-37; Sambrook *et al.*, 2001, Molecular Cloning, A Laboratory Manual, Third Edition, Cold Spring Harbor Laboratory Press, N.Y.

The cytotherapeutic units will preferably comprise minimum numbers of preselected types of potent cells and be certified as such. As used herein,

"preselected" refers to the process of selecting the types of potent cells that are to be in the cytotherapeutic unit before it is administered. Preselecting the types of potent cells that will have a minimum number of those cells in the cytotherapeutic unit allows the cytotherapeutic unit to be tailored to a composition desired to achieve a specific therapeutic result in an individual or class of individuals. Likewise, certification as to the absence of other preselected types of cells is preferred for similar reasons.

The plurality of potent cells and of cell types present in the cytotherapeutic units of the invention are selected to render the units suitable for therapy for an indicated disease state or condition. As used herein, the phrase "selected to render" refers to the process of deciding that a cytotherapeutic unit comprising a plurality of potent cells is suitable for therapy. This decision can be based on the numbers of potent cells present in the cytotherapeutic unit. As discussed hereinbefore, the number of cells appears to be critical for the success rate of treating an individual or patient with cytotherapy. Therefore, not all cytotherapeutic units may be suitable for therapy for an indicated disease state or condition. Additionally, the types of potent cells will also aid in the decision process on whether or not a cytotherapeutic unit is suitable for therapy. Certain types of potent cells can be detrimental or beneficial to the treatment of a specific disease state or condition. Thus, the types of cells present in the unit can be another factor that is used to select a unit suitable for therapy. The criteria that are used to select a unit that is suitable for therapy is not specific to those mentioned above. Any set of criteria can be used to decide whether or not a plurality of potent cells present in a cytotherapeutic unit are selected to render the unit suitable for therapy of an indicated disease state or condition.

The present invention provides for cytotherapeutic units wherein at least some of the potent cells present in the unit are identified and counted. However, for the units to be relied upon in scientific research and to be used as a cytotherapeutic the units' contents must be preferably assayed to ensure the accuracy of the identities and numbers. The assays can be done by the same group, individual, or machine that had determined the identities and the numbers of at least some of the potent cells in the cytotherapeutic units. However, the assays can be performed by a different individual, group, or machine that had determined the identities and numbers of some of the potent cells. In some embodiments, only one assay needs to be performed to ensure the accuracy of the identities and the numbers. In some other embodiments, at least 2, at least 5, or at least 10 assays are performed to ensure the accuracy of the identities and the numbers of the potent cells. The types of assays to be done can be the same assay that was used to determine the numbers and the identities previously. In some other embodiments, different assays are used to ensure the accuracy of the numbers and identities of some of the potent cells. Some assays that can be used to ensure the accuracy include, without limitation, ELISA, FACS, western blot, and the like.

In some other embodiments, the provider of the unit certifies the accuracy of the assay. As used herein, the term "provider" refers to an individual, business, or facility that is providing the cytotherapeutic unit to the individual that is using the unit. In some embodiments, the certification comprises a written statement indicating that the assay was performed correctly and that the results are correct. In some other embodiments, the certification comprises results from an assay done on a positive control to show that the assay was functioning properly. In some other embodiments, the certification comprises both the results of the positive control and a written

statement that the assay was functioning properly. In some further embodiments, the certification comprises a list of the types of potent cells that have been excluded from the cytotherapeutic unit. In some further embodiments, the certification comprises a list of at least some of the types of potent cells that are contained in the cytotherapeutic unit. In some embodiments, the certification comprises the numbers of all the cells. In some embodiments, the certification further comprises the quantity of at least some of the specific cell types. In some other embodiments, the certification comprises a list of the types of at least some of the potent cells that have been added to the unit to supplement the potent cells so that the unit comprises minimum numbers of potent cells.

The present invention also provides for kits for the treatment of a person suspected of having a disease state or condition comprising a cytotherapeutic unit comprising a plurality of potent cells with the content of the unit being known with respect to the identities and numbers of at least some of the potent cells. Additionally, the cytotherapeutic unit is assayed to ensure the accuracy of the identities and numbers of the potent cells. The kits further comprise a certification of the accuracy of the assay. In some embodiments, the kits comprise a cytotherapeutic unit having minimum numbers of identified potent cells and a certification of the potent cell composition of the unit. In some other embodiments, the kits comprise cytotherapeutic units that have at least one cell-type that has been excluded.

The present invention also provides for methods of treating a disease state or condition in a mammal. The methods comprise administering to the mammal a therapeutically effective amount of a composition comprising a cytotherapeutic unit comprising potent cells, wherein some of the potent cells are known with respect to their identities and numbers. The unit is also assayed to ensure the accuracy of the

identities and the numbers. In some other embodiments, the cytotherapeutic unit comprises minimum numbers of preselected types of potent cells.

A therapeutically effective amount for a mammal can vary, but for example could be approximately 0.01 cytotherapeutic units/kg to 100 units/kg. The cytotherapeutic unit can be administered rapidly or slowly to the mammal. In some embodiments, the cytotherapeutic unit is administered at a rate of approximately 0.01 µl /minute, and in other embodiments, the unit is administered at a rate of approximately 100,000 ml/minute. The unit can be administered, for example, intravenously, subcutaneously, intramuscularly, orally, or rectally. In some embodiments, the unit is administered multiple times to the mammal at different times. In some other embodiments, cytotherapeutic units derived from different sources or different individuals are administered to the mammal.

The potential uses for cytotherapeutic units are limitless, but some examples of disease states or conditions that cytotherapeutic units can be used to treat include cancer, acute leukemia, chronic leukemia as well as other cancers presently treated with bone marrow or cord blood cell transplants, myelodysplastic syndrome, stem cell disorder, myeloproliferative disorder, lymphoproliferative disorder, phagocyte disorder, liposomal storage disorder, histiocytic disorder, inherited erythrocyte abnormality, congenital (inherited) immune system disorder, inherited platelet abnormality, plasma cell disorder, Lesch-Nyhan Syndrome, Cartilage-Hair Hypoplasia, Glanzmann Thrombastenia, osteoporosis, breast cancer, Ewing Sarcoma, neuroblastoma, renal cell carcinoma, lung cancer, Alzheimer's disease, liver disease, hepatitis, Parkinson's disease, vision loss, memory loss, and the like.

The cytotherapeutic units may be optimized for enzyme replacement therapy to treat specific diseases or conditions, including, but not limited to, lysosomal storage

diseases, such as Tay-Sachs, Niemann-Pick, Fabry's, Gaucher's, Hunter's, and Hurler's syndromes, as well as other gangliosidoses, mucopolysaccharidoses, and glycogenoses. The cytotherapeutic units in this case may be certified that the cells have been assayed to contain the desired number of cells capable of producing the necessary enzyme. Said unit may contain either allogeneic cells containing the functional endogenous gene of the desired enzyme, autologous cells containing exogenous copies of the desired gene or a combination of both.

In other embodiments, the cells may be used as autologous or heterologous transgene carriers in gene therapy to correct inborn errors of metabolism such as adrenoleukodystrophy, cystic fibrosis, glycogen storage disease, hypothyroidism, sickle cell anemia, Pearson syndrome, Pompe's disease, phenylketonuria (PKU), Tay-Sachs disease, porphyrias, maple syrup urine disease, homocystinuria, mucopolypsaccharidoses, chronic granulomatous disease, and tyrosinemia or to treat cancer, tumors or other pathological conditions.

The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that present invention is not entitled to antedate such publication by virtue of prior invention.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication,

patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

EXAMPLE 1

An adult having acute myelogenous leukemia (AML) is in need of hematopoetic reconstitution by way of a cell transplant. The patient undergoes traditional chemotherapy followed by conventional preparation for transplant as determined by the patient's health care provider but includes destroying the diseased bone marrow. The patient's weight is determined. Appropriate HLA typing has been done by conventional methods. Based on these parameters, which include the disease to be treated, the patient's body weight and HLA matching, the transplanter requests and is provided with a cytotherapeutic unit comprising a plurality of potent nucleated cells; the content of said unit being known with respect to the identities and numbers at least some of said plurality; the unit being assayed to ensure the accuracy of said identities and numbers, which is certified. In particular, the unit is certified to contain about 1.4×10^7 nucleated cells per kilogram of the patient's body weight. Additional certified information includes HLA information. Because the patient suffers from AML, the cytotherapeutic unit contains no less than one (1) percent of CD34+ of the total nucleated cells and no less than 2.5 percent CD8+ cells to minimize graft versus tumor effect. In this case the transplanter requests twice the total number of cells needed for transplant (1.4×10^7 nucleated cells multiplied by the patient's weight in kilograms x2). The transplanter requests the 1x amount just prior to the transplant in order to have the number of cells suitable for this transplant. The second half of the cells is to be shipped in the event that a second transplant becomes necessary. Accordingly, the second cytotherapeutic unit is the same as that to be used in the initial transplant. Alternatively, the transplanter may request, based on alterations in

the patient's weight, severity of disease or even changes in recommended treatment, that the second cytotherapeutic unit be altered in the appropriate manner (increased number of CD34 positive cells, etc.) and certified. The transplant is performed in the same manner conventionally used by the transplanter.

EXAMPLE 2

A child having sickle cell anemia is in need of a cell transplant. It is determined that 1.7×10^7 nucleated cells per kilogram of body weight of the child is needed. Appropriate HLA typing is done by conventional methods. It is determined that the cytotherapeutic unit must have no less than 1% CD34+ cells of the total nucleated cells. Said CD34+ cells are further described in a ratio of 2:1 as CD34+/CD33+: CD34+/CD33$^-$ A cytotherapeutic unit having these parameters is provided. This unit comprises cells derived from cord blood as well as pluripotential placental cells such as those described in WO 02/064755, which are derived in the manner described in WO02/064755. The ratio of CD34+/ CD33+ cells is 2:1 to CD34+/ CD33-, a fact which is ascertained by assay and certified as being accurate. The certified cells are determined using FACS; based on the fluorescent properties of the particles, cell surface marker-specific antibodies or ligands are labeled with distinct fluorescent labels. Cells are processed through the cell sorter, allowing separation of cells based on their ability to bind to the antibodies used. Cell surface marker-specific antibodies may be purchased from any company selling such reagents, including Becton Dickinson, for example. The transplant is performed in the same manner conventionally used by the transplanter.

EXAMPLE 3

A child suffers from adrenal leukodysplasia. It is determined that a cellular transplant is appropriate. It is determined that 2×10^7 nucleated cells (derived from cord blood by a conventional technique) per kilogram of body weight of the child is needed. Appropriate HLA typing is done by conventional methods. A cytotherapeutic unit having these parameters is provided. In particular, the unit is certified to contain no less than 0.25% of CD34+/ CD38- cells and with no less than 0.5% depletion of CD8+ cells of the total nucleated cells. The transplant is performed in the same manner conventionally used by the transplanter.